

Appl. No. : 09/272,835
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B' concl.
factors are believed to have important signaling functions in neural tissues, based in part upon the precedent established with nerve growth factor (NGF). NGF supports the survival of sympathetic, sensory, and basal forebrain neurons both *in vitro* and *in vivo*. Administration of exogenous NGF rescues neurons from cell death during development. Conversely, removal or sequestration of endogenous NGF by administration of anti-NGF antibodies promotes such cell death (Heumann, *J. Exp. Biol.*, 132:133-150 (1987); Hefti, *J. Neurosci.* 6:2155-2162 (1986); Thoenen, *et al.*, *Physiol. Rev.*, 60:1284-1335 (1980)).--

Please replace the paragraph beginning at page 1, line 25 with the following rewritten paragraph:

B²
-- Additional neurotrophic factors related to NGF have since been identified. These include brain-derived neurotrophic factor (BDNF) (Leibrock, *et al.*, *Nature*, 341:149-152 (1989)); neurotrophin-3 (NT-3) (Kaisho, *et al.*, *FEBS Lett.*, 266:187 (1990); Maisonpierre, *et al.*, *Science*, 247:1446 (1990); Rosenthal, *et al.*, *Neuron*, 4:767 (1990), and neurotrophin 4/5 (NT-4/5) (Berkemeier, *et al.*, *Neuron*, 7:857-866 (1991)).--

Please replace the paragraph beginning at page 17, line 33 and ending at page 18, line 10 with the following new paragraph:

B³
-- Glial cell line-derived neurotrophic factor ("GDNF") (Lin *et al.*, *Science*, 260:1130-1132 (1993); WO 93/06116, which are incorporated herein in its entirety), is a potent survival factor for midbrain dopaminergic (Lin *et al.*, (1993), *supra*; Strömberg *et al.*, *Exp. Neurol.*, 124:401-412 (1993); Beck *et al.*, *Nature*, 373:339-341 (1995); Kearns *et al.*, *Brain Res.*, 672:104-111 (1995); Tomac *et al.*, *Nature*, 373:335-339 (1995)), spinal motor (Henderson *et al.*, *Science*, 266:1062-1064 (1994); Oppenheim *et al.*, *Nature*, 373:344-346 (1995); Yan *et al.*, *Nature*, 373:341-344 (1995)), and noradrenergic neurons (Arenas *et al.*, *Neuron*, 15:1465-1473 (1995)), which degenerate in Parkinson's disease (Hirsch *et al.*, *Nature*, 334:345-348 (1988); Hornykiewicz, *Mt. Sinai J. Med.*, 55:11-20 (1988)), amyotrophic lateral sclerosis (Hirano, *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disease*, P. Rowland, ed. (New York: Raven Press, Inc.) pp. 91-101 (1991), and Alzheimer's disease (Marcyniuk *et al.*, *J. Neurol. Sci.*, 76:335-345 (1986); Cash *et al.*, *Neurology*, 37:42-46 (1987); Chan-Palay *et al.*, *Comp. Neurol.*, 287:373-392 (1989)), respectively. Based on mice genetically engineered to lack GDNF,

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β^3
corel. additional biological roles for GDNF have been reported: the development and/or survival of enteric, sympathetic, and sensory neurons and the renal system, but not for catecholaminergic neurons in the central nervous system (CNS) (Moore *et al.*, *Nature* 382:76-79 (1996); Pichel *et al.*, *Nature* 382:73-76 (1996); Sanchez *et al.*, *Nature* 382:70-73 (1996)). Despite the physiological and clinical importance of GDNF, little is known about its mechanism of action.--

Please replace the paragraph beginning at page 51, line 21 with the following rewritten paragraph:

β^4 -- Primers containing sense sequence GCCCGCGACCTCCACTGCTG (designated gfrp1; SEQ ID NO: 22) and antisense sequence CTGTGGGGAGCGGCGGCG (designated gfrp2.r.c; SEQ ID NO: 23) were used to generate a 671 bp hybridization probe from the mouse GFR α 3. Primers containing sense sequence CCTGAACCTATGGTAACTGG (SEQ ID NO: 24) and antisense sequence ACCCAGTCCTCCCTACC (SEQ ID NO: 25) were used to generate a 378 bp hybridization probe from the mouse GFR α 3.--

Please replace the paragraph beginning at page 53, line 10 with the following rewritten paragraph:

β^5 -- In an alternative technique, mammalian GFR α 3 may be introduced into 293 cells transiently using the dextran sulfate method described by Sompayrac *et al.*, *Proc. Natl. Acad. Sci.*, 78:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 μ g pRK5-GFR α 3 DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20 % glycerol for 90 second, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 μ g/ml bovine insulin and 0.1 μ g/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed mammalian GFR α 3 can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography. --

Please replace the paragraph beginning at page 54, line 17 with the following rewritten paragraph:

B⁶
--Alternatively, expressed poly-his tagged GFR α 3 can be purified by Ni²⁺-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Ruppert *et al.*, *Nature* 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl₂; 0.1 mM EDTA; 10% Glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50mM phosphate, 300 mM NaCl, 10% Glycerol, pH 7.8) and filtered through a 0.45 μ m filter. A Ni²⁺-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to a baseline A₂₈₀ with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50mM phosphate; 300 mM NaCl, 10% Glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A₂₈₀ baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. On mL fractions are collected and analyzed by SDS-PAGE and silver staining or western blot with Ni²⁺-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His₁₀-tagged GFR α 3 are pooled and dialyzed against loading buffer.--

Please cancel the Sequence Listing numbered as pages 1-34 immediately following the Abstract of the Disclosure, and replace it with the attached substitute Sequence Listing, pages 1-26. ✓

In the Claims:

Please cancel claims 1-15, without prejudice.

Please add the following new claims:

B⁸
--66. An isolated nucleic acid molecule comprising a nucleic acid encoding a polypeptide having at least 80% sequence identity with amino acid residues 27 to 374 of the native sequence murine GFR α 3 polypeptide of Figures 1A-B (SEQ ID NO: 5), and having the ability to regulate peripheral neuronal function.